Original Article Combination of culture and dyeing conditions on cloth color dyed with mixed fungi dyes

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Abstract

This study aimed to examine the dyes production of mixed *Aspergillus* and *Paecilomyces* on different culture conditions and evaluate the combination between culture and dyeing conditions on cloth color by the fungal dyes. The different media consist of mineral salt glucose (MSG) medium and mineral salt sucrose (MSS) medium were evaluated. Additionally, different culture conditions such as C and N sources, incubation temperature, and pH of the media were evaluated together with variation of dyeing conditions including pH and mordants. The result showed that different culture conditions on media and the combination of those with dyeing conditions produce distinction colors on the fabric. The variation of dyed colors on the fabric was related to the dyes concentration produced by combination treatment of culture condition and dyeing pH. However, the effect was not observed on the combination between culture condition and mordants. Combination of culture and dyeing conditions on cloth color dyed with mixed fungi dyes generated various cloth colors, adding a color variation on textile dyeing.

Keywords: cloth (fabric/textile), color, culture condition, dyeing condition, fungal dyes

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Introduction

Preference of natural dyes has been a concern due to the harmful effect of synthetic dyes both to human health and the environment. Plant, animal, insects, microorganism, and mineral sources (ores) have been investigated for extraction of dyes and pigments (Basant and Jahan, 2016). According to Dufosse et al. (2014), filamentous fungus is one of the potential organisms to synthesize and secrete an extraordinary pigment molecule such as quinone, monascins, violacein, and indigoids. It has been reported that dyes production by mixed Aspergillus and Paecilomyces and its coloring on cotton cloth increased by optimizing culture condition (Suciatmih, 2019). However the effect of culture condition such as medium, incubation temperature, and medium pH that combined with dyeing condition such as mordant and dyeing pH to stain cloth has not been determined.

Bhattacharyya and Jha (2011) reported that different media affect biomass and bioactive metabolite production. Unlike other complex media, malt extract medium significantly increase the growth and pigment production of three isolates, *Aspergillus keveii* (CML 2968), *Penicillium flavigenum* (CML 2965), and *Fusarium* sp. (CML 2969) (Souza et al., 2016). While potato dextrose broth produces the best yield of biomass and pigment production of *Aspergillus terreus* compared to other broth media (Akilandeswari and Pradeep, 2017). Soluble starch and peptone as C and N sources, respectively, were able to induce *Penicillium* sp. the production of red pigment

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Research Center for Biology, Indonesian Institute of Sciences, Cibinong Science Center, Cibinong, West Java, Indonesia Phone: +622187907636 Fax: +622187907612 e-mail: suciatmih2008@yahoo.ca (Gunasekaran and Poorniammal, 2008). On the other hand, the addition of glucose as a C source and NaNO₃ as an N source optimize pigment secretion of *Pezicula* sp. BDE9/1 (Dey et al., 2016).

Temperature and pH are environmental condition that known has strong effect on biosynthesis of metabolite including pigment. Ogbonna (2016) reported that the pH on growth medium affects most aspects of the production process such as the cellular metabolism and nutrient absorption and utilization by the organism, while temperature affects the membrane fluidity which affect the uptake of nutrients and product excretion by microorganisms. The optimal incubation temperature and medium pH for pigment production of *Pezicula* sp. BDE9/1 were found to be 23 °C and pH 5 (Dey et al., 2016) and *Penicillium* when initial pH of culture medium set at pH 9.0 and 30 °C (Gunasekaran and Poorniammal, 2008).

Different shades and tones might be obtained from a single dye source by application of the pH value of its bath (Wang et al., 2014; Ren et al., 2016; Suciatmih and Yuliar, 2018) and mordant (Sangeetha et al., 2015; Suciatmih and Hidayat, 2017; Suciatmih and Yuliar, 2018). The effect of the dyeing pH can be attributed to the correlation between the pigment structure and cotton cloth. The anion of the pigment has complex characters, and when it bound on the fiber, with ionic forces, the ionic attraction would increase the dye ability of the cloth (Nagia et al. in Velmurugan et al., 2010). In contrast, at above pH 5 (high pH), the ionic interaction between the pigment and cotton cloth decreased due to the decreasing number of protonated terminal amino groups, thus lowering its dye ability (Kamel et al. in Velmurugan et al., 2010). From Satyanarayana and Chandra (2013), mordants help in the binding of colors to the fiber by forming a chemical bridge between the two.

In this study, we aim to examine the dyes production of mixed *Aspergillus* and *Paecilomyces* on different culture conditions and evaluate the effect of different cul-

Methods

Selection of culture media

Inoculation process was performed as our previous method (Suciatmih, 2019). Briefly, each five mycelial prints were inoculated into three different media (per liter of distilled water) separately: 1) mineral salt glucose (MSG) medium (Backer and Tatum, 1998) contained: 20 g glucose, 0.848 g NaNO₃, 0.3 g KCl, 0.165 g MgSO₄.7H₂O, 0.1 g NaH₂PO₄, 0.04 g CaCl₂.2H₂O, 0.0057 g H₃BO₃, 0.005 g FeSO₄.7H₂O, 0.0044 g ZnSO₄.7H₂O, 0.0031 g MnSO₄.H₂O, 0.0025 g Na₂MoO₄.2H₂O, 0.0004 g CuSO₄.5H₂O, and pH 6.0; 2) mineral salt glucose (MSG) medium (Cho et al., 2002) contained: 30 g glucose, 2 g yeast extract, 2 g peptone, 0.5 g MgSO₄.7H₂O, 0·46 g KH₂PO₄, 1 g K₂HPO₄, and pH 5; and 3) mineral salt sucrose (MSS) medium (Poorniammal et al., 2013) contained: 30 g sucrose, 3 g NaNO₃, 0.5 g KCl, 1 g K₂HPO₄, 1 g MgSO₄, and pH 6. All of the media were incubated at room temperature in a static way for one month in the dark place. At preliminary research, MSG medium (Backer and Tatum, 1998) was choosen for pigment production of the fungal.

C sources at a concentration of 2% (glucose, lactose, and sucrose) and N sources at a concentration of 0.09% (KNO₃, monosodium glutamate, NaNO₃, and yeast extract) were supplemented separately into MSG medium. The effect of culture conditions, including incubation temperatures (24, 27, and 30 °C) and media pH (5, 7, and 9) were also evaluated on the medium using glucose as C and NaNO₃ as N sources.

ture conditions on media and the combination of those with dyeing conditions on cloth color by the fungal dyes.

Harvesting of dye

After incubation with different treatments, the culture was passed through five layers of cheesecloth, and the filtrate was centrifugated at 8500 rpm for 20 min as performed previously (Suciatmih, 2019). The optical density of the filtrate was determined by spectrophotometre (Shimadzu) at 530 nm for quantifying the dyes (Suciatmih, 2019). The dyes' yield was calculated as OD units (UA530).

Mordanting procedures

Natural dyes have a low coloring power that require chemicals compound (mordants) for dye fixation into the fiber, while dyeing pH can increase or decrease the dye ability to bind the fiber. To assess the combination between different culture and dyeing conditions on clothing color, the fungi producing dyes treated in different dyeing procedures such as mordant supplemented (alum, CaCO₃, and FeSO₄.7H₂O) and dyeing pH (3, 7, and 9) were analyzed.

The pre-mordanting technique was performed in this study (Suciatmih, 2019). Briefly, cloth (4 cm \times 4 cm or 0.24 g) was dipped in required mordanting solution 1.2% (1: 30 w/v) (material: liquor) for 30 min at 90°C; and then it dyed with the dyes at material to the liquor of 1: 30 w/v, for 30 min at 90°C and left overnight. The cloth was removed, squeezed, washed with 1% light soup, rinsed with water, and then air-dried at room temperature. The color of cloth after dyeing from each treatment was determined by matching the cloth color with the Royal Horticultural Society (RHS) color chart repeatedly (The Royal Horticultural Society, 1966). The procedures were carried out twice.

Results

Effect of different media

In this study the result showed that a mixture of *Aspergillus* and *Paecilomyces* cultures on different media produced different dye colors. For instance, the fungi grown on the MSG medium (Backer and Tatum, 1998) produced violet dyes, while on the MSG (Cho et al., 2002) and the MSS (Poorniammal et al., 2013) media produced red dyes respectively (Fig. 1).

The MSG medium (Backer and Tatum, 1998) was significantly favored for pigment production, so its medium is choosen for culture condition. The optical density of the fungal dyes cultured on the media of Backer and Tatum (1998), Cho et al. (2002), and Poorniammal et al. (2013) was 3.8850 UA/L, 0.8392 UA/L, and 0.9781 UA/L respectively.

Figure 2 showed that MSG medium (Backer and Tatum, 1998) for fungi culture produced dyes stained cloth from 155D white (K) into 70B red-purple (No. 1), while Cho et al. (2002) and Poorniammal et al. (2013) media produced dyes each stained cloth from 155D white (K) into 155C white (No. 2 & 3).



Figure 1. Fungal dyes on different media 1= Medium of Baker & Tatum (1998); 2= Medium of Cho et al. (2002); 3= Medium of Poorniammal et al. (2013)



Figure 2. Cloths dyed with the fungal dyes obtained on different media. K = control; 1= Medium of Backer & Tatum (1998); 2= Medium of Cho et al. (2002); 3= Medium of Poorniammal et al. (2013)

Combination effect of different C sources and dyeing pH on cloth colors dyed with the fungal dyes

The different C sources supplemented on the MSG medium (Backer and Tatum, 1998) combined with different dyeing pH (3, 7, 9, and control) showed various colors on the cloth (Tab. 1). The dyes changed a cloth from white into grey, greyed-purple, greyed-red, red, red-purple, and violet.

Dyes producing fungi cultured on the medium containing glucose as a C source combined with different dyeing pH produced three different cloth colors (182C greyed-red, 70B red-purple, and 88B violet). On the medium containing lactose, three diverse colors were obtained on the cloth (36A red, 69B red-purple, and 88D violet); while sucrose supplemented medium generated four different colors on the fabric (201B grey, 186B greyed-purple, 182C greyed-red, and 70B red-purple). The optical density of the dyes using glucose, lactose, and sucrose was 3.5931 ± 0.0655 UA/L, 3.0735 ± 0.1130 UA/L, and 4.2500 ± 0.0721 UA/L respectively.

Different C sources (glucose, lactose, and sucrose) contained on the medium combined with the dyeing at pH 3 was obtained the reddish cloth colors, 182C greyed-red and 36A red respectively.

Combination effect of different N sources and dyeing pH on cloth colors dyed with the fungal dyes

The combination of N sources (KNO₃, monosodium glutamate, NaNO₃, and yeast extract) on the medium (glucose as a C source) and dyeing pH (3, 7, 9, and control) composed of multiple shades on the cloth (Tab. 2). Greyed-orange, greyed-purple, greyed-red, red-purple, violet, and yellow-orange appear on the white cloth dyed.

The dyes obtained from the medium containing KNO₃ as an N source combined with different dyeing pH gener-

ated three distinct cloth colors (174D greyed-orange and 88C & D violet). Three different colors produced by monosodium glutamate supplementation (174D greyed-orange; 186A greyed-purple; and 70B red-purple) were obtained. The NaNO₃ supplementation also produced 182C greyed-red, 70B red-purple, and 88D violet; while the yeast extract yielded 20C & D yellow-orange and 88D violet. The optical density of the dyes using KNO₃, monosodium glutamate, NaNO₃, and yeast extract was 3.9701 \pm 0.0146 UA/L, 4.041 \pm 0.142 UA/L, 4.070 \pm 0.044 UA/L, and 4.0693 \pm 0.0950 UA/L respectively.

Different N sources (except yeast extract) at pH 3 produced the reddish cloth colors, including 174D greyed-orange and 182C greyed-red.

Combination effect of different incubation temperatures and dyeing pH on cloth colors dyed with the fungal dyes

Different incubation temperatures (24, 27, and 30 °C) of the medium (glucose as C and NaNO₃ as N sources) and different pH condition (3, 7, 9, and control) produced various colors on the cloth (Tab. 3). In case the white fabric is dyed, it produced greyed-orange, greyed-purple, red-purple, and violet.

Dyes producing fungi cultured on the medium at the incubation temperature of 24 °C with different dyeing pH produced four different colors on the cloth (174D greyed-orange, 186B greyed-purple, and 87C & 88D violet). While at 27 °C, it produced three different cloth colors (174D greyed-orange, 70C red-purple, and 86A violet), while at 30 °C, 173D greyed-orange and 88D violet was obtained. The optical density of the dyes at different incubation temperature (24, 27, and 30 °C) was 4.4244 ± 0.1533 UA/L, 3.56 ± 0.0112 UA/L, and 2.3230 ± 0.0374 UA/L respectively.







As in the C and N (except yeast extract) sources, the dyes obtained from the medium at different incubation temperatures combined with the pH 3 generated the reddish cloth colors (greyed-orange).

Combination effect of different medium pH and dyeing pH on cloth colors dyed with the fungal dyes

The combination between different pH (5, 7, and 9) of the medium (glucose as C and NaNO₃ as N sources) and different dyeing pH (5, 7, 9, and control) produced various shades on the cloth (Tab. 4). White cloths dyed with the dyes turn greyed-orange, greyed-purple, greyed-red, purple, red-purple, violet, and violet-blue.

The dyes obtained from the medium at the pH 5 incubation combined with different dyeing pH produced four different colors on the cloth (174C greyed-orange, 70C red-purple, and 87C & 88D violet). Four distinct cloth colors (174C & 173D greyed-orange, 70C red-purple, and 87C violet) were obtained at the pH 7 incubation. Moreover, a pH of 9 generated four different colors on the cloth (186B greyed-purple, 182C greyed-red, 79D purple, and 90B violet-blue). The optical density of the dyes by the media pH of 5, 7, and 9 was 3.8471 ± 0.0382 UA/L, 3.9860 ± 0.0152 UA/L, and 4.079 ± 0.0624 UA/L respectively.

The C and N (except yeast extract) sources and all incubation temperatures of the medium, the dyes obtained from the medium at all incubation pH combined with the dyeing pH 3, produced reddish colors on the cloth, 174C greyed-orange, 174C greyed-orange, and 182C greyed-red.

Combination effect of different C sources and mordants on cloth colors dyed with the fungal dyes

Different C sources (glucose, lactose, and sucrose) on the medium (NaNO₃ as an N source) combined with various mordants (alum, CaCO₃, FeSO₄.7H₂O, and control) generated multiple colors on the cloth (Tab. 5).

Dyes producing fungi on the medium containing glucose as a C source with different mordants, produced three distinct colors on the cloth, 200C brown, 70B redpurple, and 87A violet. Lactose produced 199D greyedbrown, 69B red-purple, and 88D violet, while sucrose showed 201A grey, 186B greyed-purple, and 79D purple. The optical density of the dyes by glucose, lactose, and sucrose was 3.5931 ± 0.0655 UA/L, 3.0735 ± 0.1130 UA/L, and 4.2500 ± 0.0721 UA/L respectively.

Combination effect of different N sources and mordants on cloth colors dyed with the fungal dyes

The combination between various N sources (KNO₃, monosodium glutamate, NaNO₃, and yeast extract) on the medium (glucose as a C source) and different mordants (alum, CaCO₃, FeSO₄.7H₂O, and control) produced various shades on the cloth (Tab. 6). The dyes changed a cloth from white into grey, greyed-brown, greyed-purple, purple, red-purple, violet, and yellow-orange.



The dyes obtained from the medium containing KNO₃, as an N source, with different mordants produced two different colors on the cloth (88C & D violet). The monosodium glutamate produced four different cloth colors, including 201B grey, 186B greyed-purple, 79D purple, and 70B red-purple. Additionally, the NaNO₃ also produced 79D purple, 70B red-purple, and 87A violet, while the yeast extract yielded 199D greyed-brown, 88D violet, and 20C yellow-orange. The optical density of the dyes by KNO₃, monosodium glutamate, NaNO₃, and yeast extract was 3.9701 \pm 0.0146 UA/L, 4.041 \pm 0.142 UA/L,

 4.070 ± 0.044 UA/L, and 4.0693 ± 0.0950 UA/L respectively.

Combination effect of different incubation temperatures and mordants on cloth color dyed with the fungal dyes

Different incubation temperatures (24, 27, and 30 °C) of the medium (glucose as C and NaNO₃ as N sources) with some mordants (alum, CaCO₃, FeSO₄.7H₂O, and control) had various colors on the cloth (Tab. 7).

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White cloths dyed turn grey, greyed-green, greyed-purple, greyed-red, red-purple, and violet.

Dyes from the fungi cultured on the medium at the incubation temperature of 24 °C with mordants produced three different cloth colors, namely 197C greyed-green, 186B greyed-purple, and 182D greyed-red. Moreover, the incubation temperature of 27 °C had four different cloth colors, 201B grey, 182D greyed-red, 70C red-purple, and 87B violet, while at 30 °C, 88C & D violet were produced. The incubation temperature of 24, 27, and 30 °C produced the absorbance of the dye of 4.4244 ± 0.1533 UA/L, 3.56 ± 0.0112 UA/L, and 2.3230 ± 0.0374 UA/L respectively.

Combination effect of different medium pH and mordants on cloth color dyed with the fungal dyes

The combination of varying pH (5, 7, and 9) of the medium (glucose as C and NaNO₃ as N sources) with various mordants (alum, CaCO₃, FeSO₄.7H₂O, and control) produced several shades on the cloth (Tab. 8). The dyes stain the white fabric to grey, greyed-green, greyed-orange, greyed-purple, greyed-red, red-purple, and violet.

The dyes from the medium at the pH 5 with different mordants generated 201C grey, 70C red-purple, and 88C & D violet. Similarly, the incubation pH 7 produced two different colors, 197C greyed-green, and 173D greyed-orange, while a pH of 9 yielded 201A grey, 186B greyed-purple, and 182C greyed-red. The absorbance of the dyes by the media pH of 5, 7, and 9 was 3.8471 ± 0.0382 UA/L, 3.9860 ± 0.0152 UA/L, and 4.079 ± 0.0624 UA/L respectively.

Table 7. Cloth color dyed with the fungal dyes added with a combination of different incubation temperatures and mordants



 Table 8. Cloth color dyed with the fungal dyes added with a combination of different medium pH and mordants

 Medium pH / Mordants
 Dyed cloth colors



Discussion

According to Bhattacharyya and Jha (2011), the changes in some nutrients in the culture media used might influence fungal growth. From Boonyapranai et al. (2008) and Premalatha et al. (2012), the components, such as metal ions/or other micronutrients in the culture media, are suitable and facilitate enzymes work effectively, increasing the growth of metabolites and dyes production. The MSG medium of Backer and Tatum (1998) used to grow mixed Aspergillus and Paecilomyces gave the highest affinity dyes production of 3.8850 UA/L than other MSG medium (Cho et al., 2002) (0.8392 UA/L) and MSS medium (Poorniammal et al., 2013) (0.9781 UA/L). There is a significant correlation between the degree of absorbance of the pigment and the strength of the fabric color dyed. Fabrics dyed with the dyes produced by the Backer and Tatum (1998) MSG medium are stronger in color than fabrics dyed with the dyes from the Cho et al. (2002) and Poorniammal et al. (2013) media each remained white to resemble the control cloth (without dyes). Therefore, the Backer and Tatum (1998) medium is used for further investigation.

The initial pH of media of Backer and Tatum (1998), Cho et al. (2002), and Poorniammal et al. (2013) were 6, 5, and 6 respectively, while the final pH (after culture) for the fungi was 7.13, 7.16, and 7.01, correspondingly. Tudor et al. (2013) reported that fungi often changeable the pH on the medium to create conditions that are suitable for them by selective uptake and exchange of ions.

The direction of the cloth color is the color arising from the dyeing with dyes (Widihastuti, 2009). In this study, the coloring process uses the dyes from the mixed fungi grown on Backer and Tatum (1998) medium in various treatments of C and N sources, and culture conditions, such as different incubation temperatures and media pH; combined with different dyeing pH (3, 7, 9, and control) and mordants (alum, CaCO₃, and FeSO₄.7H₂O and control).

Our previors study (Suciatmih and Yuliar, 2018) obtained that a single dye source added with application of the pH value of its bath produce different colors and tones. The dyes obtained from the medium composing of different C and N sources, and at different incubation temperatures and pH; combined with different dyeing pH showed various colors on the cloth (Tab. 1, 2, 3, & 4). Similar results were reported by Suciatmih and Yuliar (2018) on woolen yarn dyed with the dyes from each fungus such as Paecilomyces lilacinus, Paecilomyces sp. strain 542, Penicillium sp. strains 1, 3, and 4UHB, Monascus purpureus, and Trichoderma harzianum with different dyeing pH (3, 6, and 9). There is a significant correlation between the degree of absorbance of the pigment and the number of colors produced from each treatment. For instance, the dyes from the medium containing sucrose with different dyeing pH produced more varied cloth colors (4 colors) than glucose (3 colors) and lactose (3 colors) respectively (Tab. 1). This is in line with the results of the dye's absorbance from the fungi cultured on the medium containing various C sources. The absorbance of the dyes using sucrose $(4.2500 \pm 0.0721 \text{ UA/L})$

was higher than glucose $(3.5931 \pm 0.0655 \text{ UA/L})$ and lactose $(3.0735 \pm 0.1130 \text{ UA/L})$, respectively.

The characteristics of color shadings on the cloth depend on the mordants type used during the dyeing proc ess (Suciatmih and Hidayat, 2017). The different C and N sources, and culture conditions, such as different incubation temperatures and pH with different mordants generated various colors on the cloth (Tab. 5, 6, 7, & 8). The results are in accordance with our previous study (Suciatmih and Hidayat, 2017) on cotton cloth dyed with the dyes from each fungus such as Aspergillus sp. strain 1 and 2; and Penicillium sp strain 1, 3a, 3b, and 720 combined with different mordants (alum, CaCO₃, and FeSO₄.7H₂O and control). There is not a significant correlation between the degree of absorbance of the pigment and the number of colors produced from each treatment. For instance, different C sources combined with various mordant yielded the same number of cloth colors (three) (Tab. 5). The absorbance of the dyes using sucrose $(4.2500 \pm 0.0721 \text{ UA/L})$ was higher than glucose (3.5931) \pm 0.0655 UA/L) and lactose (3.0735 \pm 0.1130 UA/L), respectively. This is probably because of the influence of metal salts contained in the materials of mordants, such as Ca^{2+} of $CaCO_3$, Al^{3+} of KAl (SO₄)₂.12H₂O, and Fe²⁺ of FeSO₄.7H₂O.

Dyes producing fungi on the medium with different C and N (except yeast extract) sources; and at all incubation temperatures and pH combined with the dyeing pH 3 produced the reddish cloth colors than pH 7 and pH 9 respectively (Tab. 1, 2, 3, & 4). Our previous study (Suciatmih and Yuliar, 2018) reported that the colors might be attributed to the changes in the dyes in the dyeing process. Generally, the dyes under strong acidic conditions (pH 3) are violet reddish. However, if the pH rises above 3, the dyes gradually change from red-violet to violet. At the pH above 5, the dyes are dark violet, and with increasing pH, the color turns to bright violet. Only the fungi cultured on the medium with yeast extract produce a yellow dye. When the pH of the dye is changed to 3, the dye remains yellow so that will not produce the reddish cloth color but 20D yellow-orange.

From this study, a mixed Aspergillus and Paecilomyces cultured on the medium of Backer and Tatum (1998) produced a potential dye that can be used in the textile industry. The dyes obtained from the medium composing of different C and N sources, and at different incubation temperatures and pH; combined with different dyeing pH and mordants, generated various cloth colors, adding a color variation on textile dyeing. Different pH and N sources of the medium combined with different dyeing pH each produced the same number of cloth colors. Moreover, sucrose and incubation temperature at 24 °C of the medium combined with different dyeing pH produced more varied cloth colors than other C sources and incubation temperatures. The dyes obtained from the medium containing all C and N (except yeast extract) sources, and at different incubation temperatures and pH produced the reddish cloth colors in case it was combined with the dyeing pH of 3.

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